Copitarsia decolora (Lepidoptera: Noctuidae) Larvae Escaping from Discarded Asparagus: Data in Support of a Pathway Risk Analysis

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ABSTRACT This research was undertaken to gather data in support of an assessment of the likelihood that *Copitarsia decolora* (Guenée) (Lepidoptera: Noctuidae), a pest of asparagus, *Asparagus officinalis* L., and other crops, could escape from the pathway followed by asparagus from the field to the consumer. Asparagus that is destroyed by cooking and consumption, being run through a trash compactor or garbage disposal, or being buried in a landfill probably cannot support development of *C. decolora* larvae. Much asparagus is discarded in dumpsters, however, and the time between disposal and removal to the landfill provides an opportunity for *C. decolora* to escape into the environment. Results of this study indicate that *C. decolora* cannot survive to the pupal stage on rotten asparagus, and survival on dried asparagus is low. However, larvae can survive at least 1 wk on both types of deteriorating asparagus held at 23.5°C. In field trials, a small percentage of *C. decolora* larvae crawled out of a dumpster filled with asparagus after 1 wk.

KEY WORDS *Copitarsia decolora*, Noctuidae, risk assessment, pathway analysis

Copitarsia decolora (Guenée) (Lepidoptera: Noctuidae) belongs to a genus of moths that is distributed from central Mexico south to southern South America. Some species are considered pests in their native range (Cortes et al. 1972, Artigas and Angulo 1973, Arestegui 1976, Apablaza 1984, De la Maza 1986, Arce de Hamity and Neder de Roman 1992, Lopez 1996, Lamborot et al. 1999), and they are of regulatory concern to the United States (USDA 2003), where they are not known to occur. Eggs and larvae of these potential pests can arrive in the United States on cut flowers and vegetables; 7,434 members of the genus *Copitarsia* were intercepted at U.S. ports of entry from 1985 to April 2000 (USDA, Port Information Network internal database). Several pest risk assessments have suggested that *Copitarsia* species pose a high risk to U.S. agriculture (Cave and Redmond 1997a,b; USDA–APHIS–PPQ–BATS 1997), but Gould et al. (2000) and Venette and Gould (2006) concluded that the availability and quality of data did not support an accurate assessment of risk. In particular, it was unclear whether immature insects could survive shipment from the field to the final consumer and whether there would be opportunities to escape from the shipment pathway.

A high rate of interception of *C. decolora* eggs on asparagus, *Asparagus officinalis* L., from Perú led to mandatory fumigation of all asparagus from that country (USDA 2003), and we have been studying this pathway in detail. The response of *C. decolora* to temperature (Gould et al. 2005) and soil moisture, survival after shipment, and host range have been determined as components of a pathway risk assessment. What remained unknown was the probability that *C. decolora* could escape from the pathway followed by asparagus from the field to the final consumer.

Asparagus grows very quickly and is harvested within 2–4 d after it emerges from the soil (based on Dean 1999). The *C. decolora* found on asparagus are, therefore, very young and mostly in the egg stage. The asparagus is harvested several times per day, is quickly chilled at the packing plant, and is kept at temperatures below 7.8°C, the threshold for egg development (Gould et al. 2005), during shipment. Most *C. decolora* eggs are consumed along with the asparagus because fresh asparagus is imported for consumption; however, not all asparagus is consumed. Surveys of importers (census of importers of asparagus from Perú), wholesalers (18 responded), and retailers (supermarkets—census of all supermarket chains in California plus 11 independent stores) revealed that on average importers discard 1% of the asparagus they process, 2.3% is discarded by wholesalers, and 7.7% by retailers (J.R.G., unpublished data). When eggs are discarded along with unsalable or poor-quality asparagus, there is an as yet undetermined risk that these eggs could hatch and that the larvae could escape, find a host plant, and potentially start a reproducing population. The probability of escape from discarded asparagus was a critical gap in the data necessary to assess the risk of establishment by *C. decolora* and is addressed in this study.

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The importance of quantifying escape from the garbage pathway has been acknowledged in other risk assessments (i.e., Auclair et al. 2005); however, experimental data were not available and escape rates were set "arbitrarily." To our knowledge, this study is the first designed to quantify the probability that a potential pest could escape from discarded produce. Asparagus is kept very cold (1–4°C) during shipment because it deteriorates rapidly at warmer temperatures (Sandsted et al. 2005). Laboratory studies were conducted to determine how long *C. decolora* larvae could survive on discarded, deteriorating asparagus that became dried or rotten. Field studies also were designed to ascertain whether larvae could or would crawl away from a deteriorating food source.

This study was designed to simulate disposal of asparagus in a dumpster or garbage can. For the purpose of pathway analysis, it can be assumed that *C. decolora* eggs on asparagus that is compacted, ground, or incinerated could not escape to establish a population, and eggs would be destroyed if the asparagus was consumed. Commercial compost heaps are designed to speed up deterioration of the vegetation and are unlikely to promote survival of *C. decolora* larvae. Most homeowners probably remove and discard the hard lower end of the asparagus; however, *C. decolora* eggs are found on the top one-third of the asparagus spear, and we have never observed them on the bottom one-third, which would be discarded by homeowners. In addition, the density of *C. decolora* eggs on asparagus spears is very low, and preliminary assessments of risk at the grocery store level, where much more asparagus is discarded than by a homeowner, show that the risk of a male and female larva leaving the garbage stream is so low that it is not measurable. The risk of getting a male and female larva at the homeowner level would be even lower because of the amount of asparagus involved and is not considered in this study. Asparagus discarded in a garbage can or dumpster at a commercial facility would therefore constitute the step of the pathway where larvae would be most likely to escape, and we chose to simulate this step in the pathway.

Materials and Methods

Survival of *C. decolora* Larvae on Dried and Rotten Asparagus. Laboratory studies were conducted to determine survival of newly emerged *C. decolora* larvae when placed on asparagus that was allowed to dry or rot. This experiment was conducted at a high-security quarantine laboratory of the United States Department of Agriculture–Animal and Plant Health Inspection Service in Bourne, MA. *C. decolora* larvae were reared on high wheat germ diet (Bell et al. 1981) in a walk-in environmental chamber at 23°C and 73% RH with a photoperiod of 14:10 (L:D) h. Adults were reared in 18-cm-diameter by 17-cm-high cardboard chambers and provided a liquid diet of orange Gatorade (The Gatorade Company, Chicago, IL). Voucher specimens are deposited in the Lepidoptera collection at the National Museum of Natural History, Smithsonian Institution, Washington, DC.

To simulate asparagus that was discarded in plastic bags or on the bottom of a dumpster or garbage can, where the asparagus would rot, spears were placed in sealed 15- by 9.5- by 5-cm Rubbermaid (Newell Rubbermaid Inc., Atlanta, GA) containers. Asparagus under these conditions quickly lost cellular structure and produced much liquid in the container. Asparagus was placed in similar containers with a 12- by 7-cm hole in the lid covered with fine organdy (mesh size 0.25 mm) to simulate asparagus that was allowed to dry out, as one might expect if it were on the top of a dumpster or garbage can. Asparagus that was allowed to dry also supported the growth of fungi. The control treatment consisted of containers where the asparagus spears were replaced every 2–3 d. One neonate larva was placed in each container and held at 23.5°C (relative humidity averaged 57, 41, and 24% during trials 1, 2, and 3, respectively). Five asparagus spears, 11 by ≈11.3 mm in diameter at the base (sufficient food for development through pupation), were placed in each container. Every 2–3 d, the survival of each individual, whether or not it had become a pupa or an adult, and the percentage of the total surface of the asparagus spears that was dried or rotten were recorded. Twenty individuals were subjected to each treatment, and the experiment was repeated three times on 20 October 2003, 15 December 2004, and 1 February 2005. The number of days before 100% of the asparagus had deteriorated and the number of days to death (for individuals that died before the adult stage) were compared using a general linear model (PROC GLM, SAS Institute 1990). Multiple comparisons were made using the Ryan–Einot–Gabriel–Welsch multiple F-test.

Escape of *C. decolora* Larvae from Garbage Cans. *C. decolora* was reared at the Laboratorio de Entomología de La Facultad de Ciencias Biològicas de la Universidad Nacional Mayor De San Marcos, Lima, Perú. The colony was reared under average environmental conditions of 20.0 ± 2°C and 66.1% RH (range 53.4–81.6%). The larvae were fed asparagus foliage that was disinfected with 6% hypochlorite solution. Pairs of adults were placed in 0.5-liter plastic containers covered with organdy. The sides of the cage were covered with brown Kraft paper (no. 2; 50-lb weight) on which the females deposited eggs. Adults were fed a mixture of honey, pollen, and water in a 1:1:4 ratio contained in 5-ml plastic containers with cotton wicking.

The eggs used for this experiment were the F2 generation and were collected every 3 d. For each replicate, 100 pieces of Kraft paper with 10 eggs each were cut from the larger sheet (1,000 eggs per replicate) and an additional 200 eggs were placed in 23- by 35- by 10-cm plastic boxes to monitor percentage of hatch. The larvae hatching from control eggs were reared on asparagus foliage in the laboratory to estimate the timing of molting to the pupal and adult stages.

Asparagus spears for the study were donated by companies that export asparagus from Perú to the

United States. Asparagus from several companies was combined before being used in the study. For each replicate, 35–40 kg of asparagus was placed in an 80-cm tall by 64-cm-diameter plastic garbage can. One-tenth of the asparagus was placed on the bottom of the garbage can, and 10 pieces of Kraft paper (100 eggs) were placed on top of the asparagus. This process was repeated until there were 10 layers of asparagus and 1,000 *C. decolora* eggs. Although the density of eggs used in the experiment was greater than that found in nature, it was necessary so that we could test a sufficient number of *C. decolora* eggs to quantify small percentages. The asparagus used in this experiment could have contained some naturally occurring *C. decolora* eggs. Asparagus shipped from Perú averages 30 spears per bunch (J.R.G., unpublished data), and 70–80 bunches were put in each garbage can. Given an average of 0.014 eggs per spear (J.R.G., unpublished data from a sample of >1 million spears visually examined at Peruvian packing sheds), one could expect ≈33 naturally occurring eggs on the asparagus, compared with the 1,000 eggs placed in the containers.

A 20-cm space remained between the top layer of asparagus and the rim of the garbage can. This surface was covered with a yellow polyethylene plastic sheet that was coated with Tangle Trap Insect Trap Coating (The Tanglefoot Company, Grand Rapids, MI) to catch any neonate larvae attempting to crawl out of the garbage can. Neonate *C. decolora* are black and were easy to see against the yellow plastic.

The garbage cans were placed in a wooden cage (3 by 2.5 by 2 m) that was covered with organdy and located outdoors at the Universidad Nacional Mayor De San Marcos, Lima, Perú. HOBO Pro Series RH/Temperature probes (Onset Computing, Inc., Bourne, MA) were placed in the garbage cans with the asparagus spears. The temperature probes were placed within the pile of asparagus and recorded temperature and humidity throughout the study. Surveys of asparagus importers, wholesalers, and retailers indicate that garbage is picked up and taken to a landfill or an incinerator at least once per week (J.R.G., unpublished data). The larvae in the experiment were therefore given 1 wk to escape from the garbage cans because garbage in a dumpster is typically collected at least once per week. After 1 wk, the sticky yellow plastic was collected, and the asparagus was placed in a pile inside of the cage. The pieces of Kraft paper were collected, and the numbers of hatched and unhatched eggs were counted. Any larvae caught in the sticky material at the top of the can were counted, collected, stored in 100% ethanol, and sent to Dr. Rebecca Simmons for identification to species by using mitochondrial DNA markers (Simmons and Scheffer 2004).

After 1 mo, the piles of asparagus were examined for larvae, pupae, and adults, with monitoring continuing for 20 d after all of the control insects had reached the adult stage. A light trap, a 55-cm-long fluorescent bulb (Philips TLT 20W/54RS) surrounded by four 35- by 55-cm yellow plastic sticky panels, was placed inside of the cage to capture any emerging adults. All adults captured in the light trap were sent to Dr. Rebecca Simmons for identification. This study was replicated 10 times between 29 May and 4 August 2004.

Table 1. Survival of *C. decolora* to the pupal and adult stages, number of days before asparagus was dried or rotten, and number of days for the larva to die for asparagus that was allowed to dry out, rot, or was replaced frequently

	Asparagus treatment[a]		
	Dried	Rotten	Fresh
% pupation (SE)	8.3 (0.5)	0.0	86.2 (0.6)
% adult eclosion (SE)	6.7 (0.4)	0.0	56.9 (0.8)
No. days to 100% dried or rotten (SE)	14.3 (0.3)a	12.6 (0.1)b	n/a
No. days to death (range)	17.5 (4–29)a	8.3 (4–16)b	41.9 (4–61)c

n/a, not applicable.

[a] Results in the same row followed by different lowercase letters are significantly different at $\alpha = 0.05$ ($n = 60$ larvae per treatment).

Results and Discussion

Survival of *C. decolora* Larvae on Dried and Rotten Asparagus. When laboratory-reared *C. decolora* were provided fresh asparagus every few days, 86.2% developed through the pupal stage and 56.9% became adults (Table 1). Over all three replicates, 6.7% of the 60 individuals became adults on dried asparagus, and no individuals survived to the pupal stage on rotten asparagus. The four individuals that developed to the adult stage on dried asparagus did so during the third replicate, when ambient relative humidity was lowest. The asparagus deteriorated significantly more quickly when allowed to rot (12.6 versus 14.3 d to 100% deterioration) ($F = 6.05$; df = 1, 75; $P < 0.0162$), and the larvae died significantly sooner as well (8.3 versus 17.5 d) ($F = 90.21$; df = 2, 118; $P < 0.0001$). Thus, larvae confined to deteriorating host plants have a very low likelihood of completing development to the adult stage. However, they can survive for at least 1 wk, even on rotting asparagus. If larvae were not confined but were allowed to search for new food sources, would or could they escape from the garbage container? The field experiment was designed to answer this question.

Escape of *C. decolora* Larvae from Garbage Cans. Calculation of percentage of hatch was possible for only 91% of the eggs because a small number (93) of the Kraft papers were too moldy to analyze. For the analysis, we assumed that all 1,000 eggs in each garbage can exhibited the percentage of hatch observed on the nonmoldy pieces of Kraft paper. Hatch averaged 90%, with a range of 85–97% (Table 2). A higher percentage of the larvae hatched in the garbage cans than in the plastic containers in the laboratory (72%).

Three days after the asparagus was placed into the garbage can, a foul smell was noted, and fungi were observed growing on the asparagus spears. The tips of the asparagus spears also were becoming dry and flaccid. On day 7, when the asparagus was removed from the garbage cans, some of the asparagus spears were dried, but most were rotten, especially those found on

Table 2. Number of first instar larvae collected in sticky bands placed at the top of garbage cans filled with asparagus and 1,000 *C. decolora* eggs

Garbage can no.	No. of eggs recovered	% hatch	No. of larvae captured	% larvae captured
1	780	86	5	0.6
2	880	85	4	0.5
3	870	89	25	2.8
4	840	92	17	1.8
5	910	90	11	1.2
6	1,000	97	2	0.2
7	990	85	3	0.4
8	920	88	15	1.7
9	917	94	8	0.9
10	963	91	18	2.0
Avg	907	90	11	1.2

the bottom of the cans. This experiment was conducted during the Peruvian winter, with temperatures ranging from 17 to 21°C. One might predict a more rapid deterioration of the asparagus during summer, reducing survival further.

On average, 1.2% of the hatched first instars were caught in the sticky band (Table 2), and all larvae were identified as *C. decolora* (R. Simmons, personal communication). It is likely that these larvae were attempting to escape the rapidly deteriorating quality of the rotting asparagus in the garbage can. Regardless of the cause, a small percentage of the larvae were able and willing to crawl out of the garbage cans.

The larvae that did not try to escape from the garbage cans presumably died or were transferred alive to the piles of asparagus. Twelve days after the asparagus was placed in the piles, some third instars were observed. We did not disturb the mounds to make a count, but we observed five to nine third instars per mound. After the piles of asparagus had been present for 3 wk, we found only one fifth instar larva, and the asparagus spears were completely rotten. Several *Drosophila* pupae and small tenebrionids were found, but no predators that could have removed individuals were noted. After eclosion of the control insects was complete, all 10 mounds of asparagus and the soil beneath them were searched, but no *C. decolora* pupae were found. The light traps were checked three times per week for the presence of adult moths, but no *Copitarsia* were ever captured. Only tenebrionid and *Musca domestica* L. adults were captured in the light traps.

In conclusion, asparagus that is discarded in a trash compactor, a garbage disposal, or a plastic bag is unlikely to support development of *C. decolora* larvae. Commercial composting of large quantities of vegetable matter involves creating conditions that heat up the compost to increase the rate of vegetable decomposition (O'Leary and Walsh 1995), conditions that do not favor the continued development of *C. decolora* larvae. In addition, no warehouses or asparagus importers report composting discarded asparagus, most municipal composting consists of yard waste and not fruits and vegetables, and only 20% of grocery chains in California reported composting vegetable waste (J.R.G., unpublished data). Even asparagus discarded in a dumpster would no longer remain viable for *C. decolora* development after it is buried in a landfill. Landfills are closely monitored by federal and state regulators to make sure that garbage is buried and are checked for compliance as frequently as twice per week (Auclair et al. 2005). Homeowners may discard the tough lower ends of asparagus spears, but *C. decolora* only lays eggs on the top one-third of the spear. That leaves the short period between when asparagus is discarded in a dumpster at a commercial warehouse or grocery store and when it is hauled to the landfill as a potential window of opportunity for *C. decolora* to escape the produce pathway. Laboratory studies, corroborated by field observations, indicated that *C. decolora* cannot survive to the pupal stage on rotten asparagus, and survival on dried asparagus is low. However, *C. decolora* larvae can survive at least a week on deteriorating asparagus, and ≈1.2% of the larvae could crawl out of the dumpster during that 1-wk window. If garbage is removed more frequently, the likelihood of escape would probably be reduced. This research only addresses the likelihood that larvae could escape from a dumpster or garbage can; it does not address the dispersal potential of those larvae, the likelihood of encountering a host plant, the probability of survival, or whether a mate and suitable oviposition substrate could be located. The probabilities of these steps occurring remain unknown and are topics for further research.

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